

**IN THE UNITED STATES PATENT AND TRADEMARK OFFICE**

APPLICANTS    Uchida *et al.*

FOR:    ENRICHED CENTRAL NERVOUS SYSTEM STEM CELL  
AND PROGENITOR CELL POPULATIONS, AND METHODS  
FOR IDENTIFYING, ISOLATING, AND ENRICHING FOR  
SUCH POPULATIONS

**Box Patent Application**

Assistant Commissioner for Patents

Washington, D.C. 20231

**PRELIMINARY AMENDMENT**

Prior to examination of the above-identified patent application, please amend the application as set forth below and consider the following remarks.

***In the Claims:***

Please cancel claims 1-22, 24-26, and 40-43, without prejudice.

Replace the pending claims with the following:

23.    (Amended)    A population of neurosphere initiating stem cells (NS-IC) produced by:
- (a)    combining a population comprising neural cells or neural-derived cells containing a fraction of NS-ICs with a reagent that recognizes a determinant on a cell surface marker recognized by monoclonal antibody AC133 or by monoclonal antibody 5E12;
  - (b)    selecting AC133<sup>+</sup> or 5E12<sup>+</sup> cells, wherein the selected AC133<sup>+</sup> or 5E12<sup>+</sup> cells are enriched in the fraction of NS-ICs as compared with the population of neural cells;
  - (c)    introducing at least one AC133<sup>+</sup> or 5E12<sup>+</sup> cell to a culture medium capable of supporting the growth of NS-IC; and
  - (d)    proliferating the AC133<sup>+</sup> or 5E12<sup>+</sup> cell in the culture medium.

27. An *in vitro* cell culture composition comprising:
- (a) a population enriched in AC133<sup>+</sup> CD45<sup>-</sup> cells or 5E12<sup>+</sup> CD45<sup>-</sup> cells; and
  - (b) a medium capable of supporting the growth the cells.
28. An *in vitro* cell culture composition comprising:
- (a) a population enriched in AC133<sup>+</sup> CD45<sup>-</sup> CD34<sup>-</sup> cells or 5E12<sup>+</sup> CD45<sup>-</sup> CD34<sup>-</sup> cells; and
  - (b) a medium capable of supporting the growth the cells.
29. An *in vitro* cell culture composition comprising:
- (a) a population enriched in AC133<sup>+</sup> 8G1<sup>-/lo</sup> cells or 5E12<sup>+</sup> 8G1<sup>-/lo</sup> cells; and
  - (b) a medium capable of supporting the growth the cells.
30. An *in vitro* cell culture composition comprising:
- (a) a population enriched in AC133<sup>+</sup> 8G1<sup>hi</sup> cells or 5E12<sup>+</sup> 8G1<sup>hi</sup> cells; and
  - (b) a medium capable of supporting the growth the cells.
31. An *in vitro* cell culture composition comprising:
- (a) a population comprising at least 50% AC133<sup>+</sup> or 5E12<sup>+</sup> neurosphere initiating cells (NS-IC) which stain positive for nestin and, in the presence of differentiation-inducing conditions, produce progeny cells that differentiate into neurons, astrocytes, and oligodendrocytes; and
  - (b) a medium capable of supporting the growth of NS-IC.
32. The composition of claim 31, further comprising a solid support to which the NS-IC are attached.
33. The composition of claim 31, wherein the population of cells has at least 70% AC133<sup>+</sup> or 5E12<sup>+</sup> cells.

34. The composition of claim 31, wherein the population of cells has at least 90% AC133<sup>+</sup> or 5E12<sup>+</sup> cells.
35. The composition of claim 31, wherein the population of AC133<sup>+</sup> or 5E12<sup>+</sup> cells is a substantially pure population.
36. The composition of claim 31, wherein the medium comprises a serum-free medium containing one or more predetermined growth factors effective for inducing multipotent neural stem cell proliferation.
37. The composition of claim 31, wherein the medium further contains a growth factor selected from the group consisting of leukocyte inhibitory factor (LIF), epidermal growth factor (EGF), basic fibroblast growth factor (FGF-2), and combinations thereof.
38. The composition of claim 31, wherein the medium further comprises a neural survival factor.
39. The composition of claim 31, wherein the NS-IC are human.

APPLICANTS: Uchida, *et al.*

### REMARKS

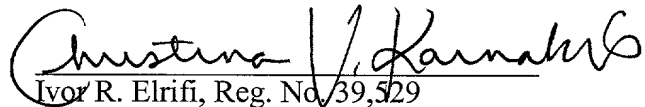
Applicants have amended claim dependent 23 to make it independent by incorporating the limitations of independent claim 22. Applications have also canceled claims 1-22, 24-26, and 40-43, without prejudice or disclaimer. No new matter has been added.

### CONCLUSION

This application is believed to be in condition for allowance, and a statement to this effect is respectfully requested. Should any questions or issues arise concerning this application, the Examiner is encouraged to contact the undersigned at (617) 542-6000.

Respectfully submitted,

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*Versions with Markings to Show Changes Made*

1.-22. (Cancelled)

23. (Amended) [A population of cells produced by the method of claim 22.] A population of neurosphere initiating stem cells (NS-IC) produced by:
- (a) combining a population comprising neural cells or neural-derived cells containing a fraction of NS-ICs with a reagent that recognizes a determinant on a cell surface marker recognized by monoclonal antibody AC133 or by monoclonal antibody 5E12;
  - (b) selecting AC133<sup>+</sup> or 5E12<sup>+</sup> cells, wherein the selected AC133<sup>+</sup> or 5E12<sup>+</sup> cells are enriched in the fraction of NS-ICs as compared with the population of neural cells;
  - (c) introducing at least one AC133<sup>+</sup> or 5E12<sup>+</sup> cell to a culture medium capable of supporting the growth of NS-IC; and
  - (d) proliferating the AC133<sup>+</sup> or 5E12<sup>+</sup> cell in the culture medium.

24.-26. (Cancelled)

40.-43. (Cancelled)

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